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Lampbrush chromosome loop-specificity of transcript morphology in spermatocyte nuclei of *Drosophila hydei*

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The transcript morphology in the lampbrush chromosome loop pairs 'threads' and 'pseudonucleolus' of the Y chromosome in primary spermatocytes of *Drosophila hydei* has been investigated by the Miller spreading technique. Each loop pair carries giant transcripts with a secondary structure characteristic of the respective loop. The lengths of the transcribed regions are in the range 500–1500 kb or even larger. The results of our experiments are discussed in the context of loop ultrastructure, molecular structure and loop function. The high degree of secondary structure may be required to assemble specifically loop-specific proteins.

Key words: gene structure/Miller spreading/spermatogenesis/transcript structure/Y chromosome

Introduction

The Y chromosome of *Drosophila hydei* forms giant lampbrush chromosome loops during its active phase in the primary spermatocyte (for review, see Hennig, 1984). Considerable progress has been made towards an understanding of the molecular structure of these lampbrush loops. Genetic experiments show that each loop pair accommodates one complementation group (Hackstein *et al.*, 1982). At the molecular level, the loops are composed of complex repeated DNA sequences (Vogt *et al.*, 1982; Lifschytz *et al.*, 1983; Vogt and Hennig, 1983; Hennig *et al.*, 1983). The presence of tissue-specific transcripts of various loops in testes has been shown by *in situ* hybridization and RNA blotting experiments (Vogt *et al.*, 1982; Lifschytz *et al.*, 1983). The loop-specific RNA fractions are present in the cells as molecules of widely variable size (Vogt *et al.*, 1982; Lifschytz *et al.*, 1983). This observation raised the question of the size of the primary transcripts. Earlier studies showed that large transcripts are associated with the Y chromosomal lampbrush chromosome loops (Meyer and Hennig, 1974; Hennig *et al.*, 1974; Glätzer and Meyer, 1981; Grond *et al.*, 1983). It is obvious that such giant transcripts cannot be isolated by current biochemical methods. Knowledge of the pattern of transcription of Y chromosomal loops relies, therefore, entirely on electron microscopic visualization of the transcription unit.

At least three of the five Y chromosomal lampbrush chromosome loops have particular structural properties (Grond *et al.*, 1984), which distinguish them from the conventional type of lampbrush chromosome loops as found in amphibian oocytes (cf. Callan, 1982). Three loops, the 'threads', the 'pseudonucleolus' and the 'clubs', accumulate proteins during the primary spermatocyte development (Grond *et al.*, 1984) which at least in part are loop-specific (Hulsebos *et al.*, 1984). How is the accumulation of loop-specific proteins achieved and what biological function is connected with this site-specificity of proteins? Answers cannot be expected before the molecular properties of these genes are better known. Here we show that the loop pairs 'threads' and 'pseudonucleolus' are giant transcription units. Each of the loops displays a highly specific pattern of transcription with respect to the secondary structure of the transcripts.

Results

The spreading of chromatin according to the method of Miller of wild-type primary spermatocytes of *D. hydei* results in pictures in which the various loops cannot be distinguished individually (Meyer and Hennig, 1974; Hennig *et al.*, 1974; Glätzer and Meyer, 1981). We therefore investigated spermatocyte nuclei with a single loop pair (see also Grond *et al.*, 1983) which should permit a direct identification of the transcript pattern of the particular loop, assuming that it is not affected by the mutant constitution. This assumption seems justified since the structure of the transcripts of the 'pseudonucleolus' (see below) is the same in different (mutant) strains. We have used spermatocyte nuclei of males with either the 'threads' or the 'pseudonucleolus' (Figures 1 and 2). These loop pairs are of special interest because of their unique structural properties (Grond *et al.*, 1984; Hulsebos *et al.*, 1984) and because of the wide range of loop mutants available (Hackstein and Hennig, 1982). Also, recombinant DNA clones of these loops have recently become available (Hennig *et al.*, 1983; Huijser and Hennig, unpublished data). The results from the spreading of both loop pairs is described below.

In general, we recognized the same three types of transcripts reported previously (Grond *et al.*, 1983). The first type is easy to identify as ribosomal transcripts (Meyer and Hennig, 1974; Glätzer, 1979). The second type includes small and widely spaced non-ribosomal transcripts as described by Grond *et al.* (1983). Transcripts of the third type are extremly large, with a high degree of secondary structure. The specific secondary structure of these large transcripts depends on the genetic constitution of the respective spermatocyte nucleus. In all cases, where only single lampbrush chromosome loop pairs are present in the nucleus due to the deletion of the residual loop-forming loci, only one characteristic conformation of the transcripts could be found. In spermatocyte nuclei without the Y chromosome we never observed such transcripts (cf. Grond *et al.*, 1983). If they were present, they should already have been detected by light microscopy. Thus, the specificity of the secondary structure of the transcripts in relation to the specific genetic constitution, and the fact that only single large loops which could carry transcripts of comparable sizes (for details see Materials and methods) are present in the spermatocyte nucleus, led us to the conclusion that the large transcripts are associated with the respective lampbrush chromosome loop pair.

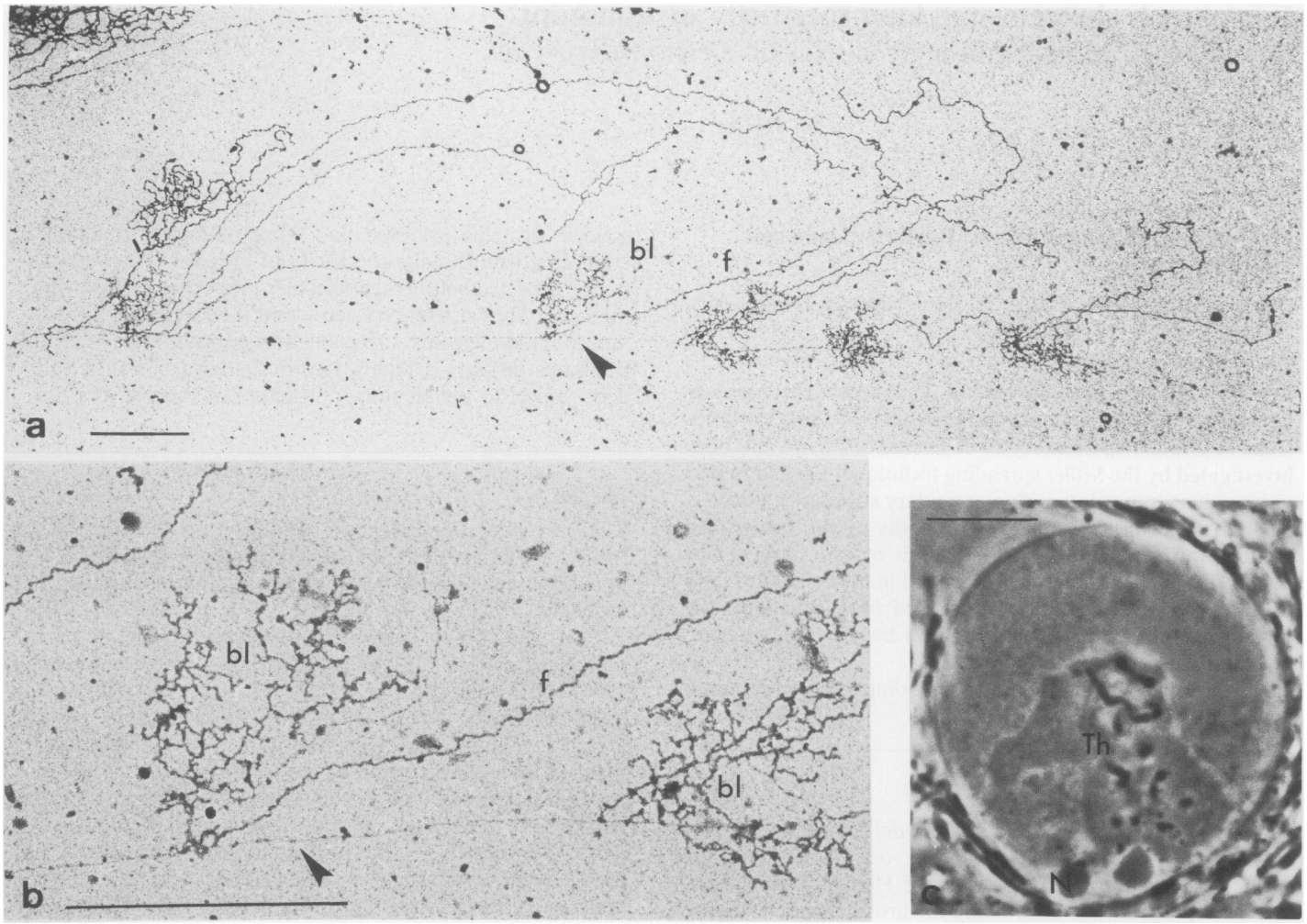

Fig. 1. Transcripts in the loop pair 'threads'. **(a)** Overview of a DNA axis with several transcripts. Arrowheads indicate the DNA axis. **(b)** Single transcript in higher magnification. Detail of **a**. **(c)** Spermatocyte nucleus of a T(X;Y)6/O male in phase contrast. Only the 'threads' (Th) are seen. N, nucleolus; bl, bush-like part; f, fibrillar part. Bars represent 1 μm in **a** and **b**, and 10 μm in **c**.

Transcription pattern of the 'threads'

In spreading experiments nuclei with only 'threads' display rather peculiar types of transcripts (Figure 1). They are composed of a bush-like portion close to the DNA axis from which extends a long fibrillar part. Not only does the secondary structure in the different parts of the transcripts differ drastically, but the diameter of the transcripts also differs. The bush-like portions of the molecules are relatively thin (3–7 nm) compared with the fibrillar portion (8–12 nm). Since the thickness of a nucleic acid molecule in a spread is related to the amount of protein associated with it, the various parts of the transcripts must carry differing amounts of protein. Moreover, in the bush-like portion many regions appear to be paired, or kept together by proteins. This is not the case for the distal fibrillar part. That the bush-like structure is a transient state of the transcripts is not entirely excluded. Our evidence on the DNA composition of the 'threads', however, indicates the presence of different types of DNA sequences within different regions of this loop pair (Huijser and Hennig, unpublished data).

The distance between the single transcripts is relatively large, although variable (1.8–5 μm) (Figure 1). It is difficult to obtain precise measurements since the exact position of the polymerases at the DNA axis is usually obscured by the bush-like part of the transcripts. From longer sections of DNA with transcripts we estimate their average distance to be ~3 μm. However, we assume that these large distances are only found in spermatocytes of stage III and IV which display a strongly reduced transcriptional activity (Hennig, 1967). In earlier developmental stages the transcript density is so high that the identification of single transcripts becomes exceedingly difficult.

From Figure 1 it is obvious that the size of the transcripts is exceptionally large. From spreads with smaller transcripts it is concluded that the complex arrays seen in Figure 2 must be considered as products of single initiation events. The longest stretch of DNA covered with transcripts as shown in Figure 1 was ~40 μm. The highly complicated structure prevents us from recognizing any gradient in the length of the transcripts. However, since all transcripts (cf. Figure 1) are of an extreme size we conclude that the total length of the transcriptional unit must considerably exceed the length of the DNA axis measured in this particular region. This conclusion is supported by a comparison with the sizes of the transcripts found along the 'nooses' (Grond *et al.*, 1983) which are transcribed from a 50 μm long loop axis.

Transcription pattern of the 'pseudonucleolus'

Transcripts of the 'pseudonucleolus' display an entirely different secondary structure which can be easily distinguished

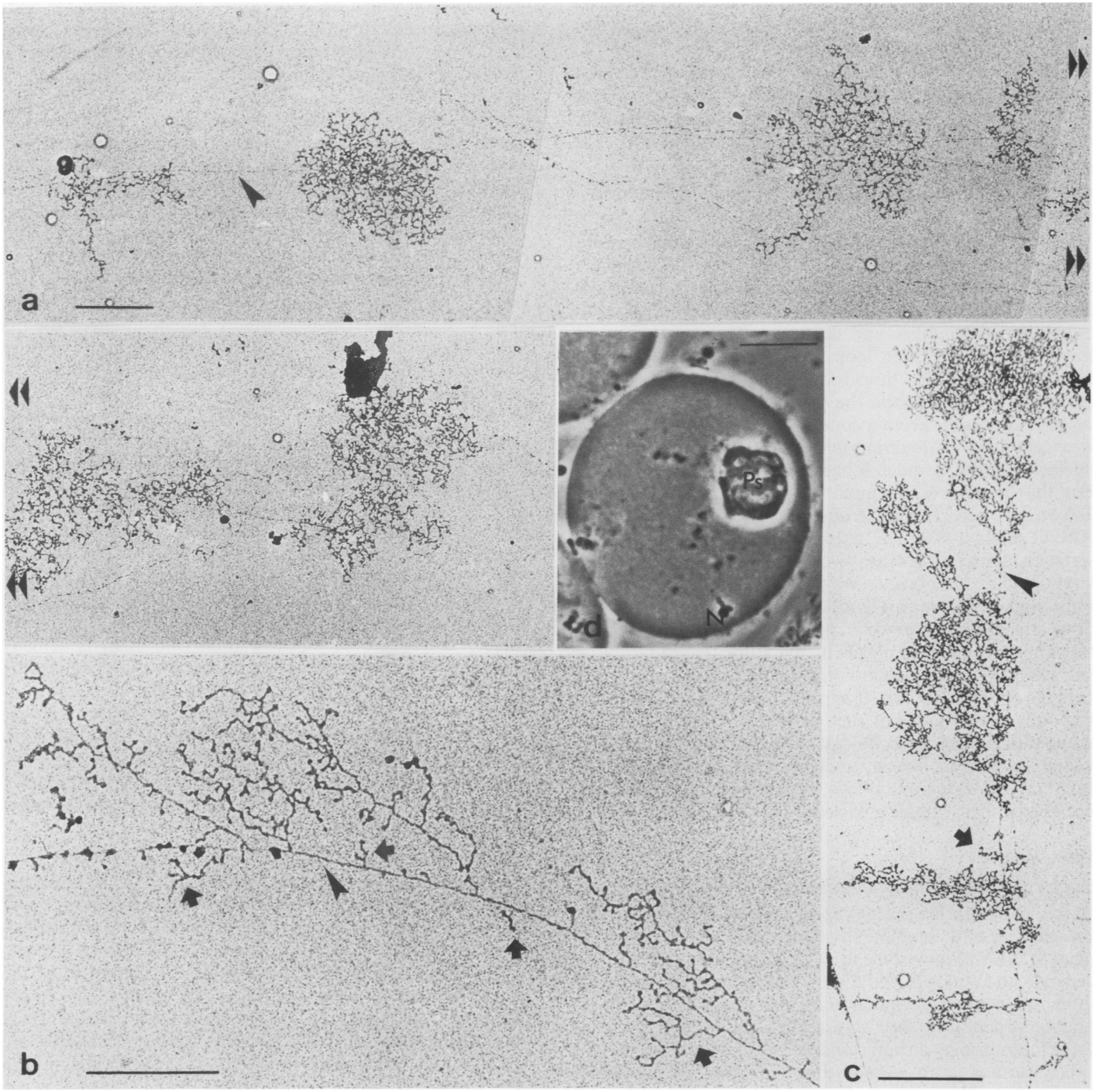

Fig. 2. Transcripts in the loop pair 'pseudonucleolus'. **(a)** Overview of a DNA axis with several transcripts. Double arrowheads indicate the positions of continuation of the figure. Long arrowhead indicates the DNA axis. **(b–c)** Transcripts at higher magnification. Note small transcripts (arrow) between the large transcripts. The gradient in the sizes of the transcripts is evident **(b)**. **(d)** Spermatocyte nucleus of a T(X;Y)74/O male in phase contrast. Only the 'pseudonucleolus' (Ps) is seen. N, nucleolus. Bars represent 1 μm in **a** and **c**, 0.5 μm in **b**, and 10 μm in **d**.

from the bush-like portions of the transcripts of the 'threads' (Figure 2). Fibrillar portions were never observed in these transcripts. Since occasionally the transcripts are bound to the DNA axis by a single polymerase molecule, we assume that the complex structures are single giant transcripts.

The distances of the single transcripts on the DNA axis are subject to an even greater variability than in the 'threads'. The smallest distances measured for 'pseudonucleolus' transcripts are <0.5 μm, the longest ~ 20 μm. Most frequently transcripts were found at distances between 1 and 9 μm. Also for this loop we assume that the transcript density is higher in spermatocyte stages with a high transcriptional activity. This is in accord with the autoradiographic data on the [^{3}H]uridine incorporation in different stages of the primary spermatocyte (Hennig, 1967). Unfortunately, it is technically not possible to select for distinct spermatocyte stages during the spreading.

A gradient in the sizes of the subsequent transcripts on a DNA axis is difficult to establish. However, this loop displays a peculiarity in its transcript pattern which has not been

observed in the other two loop pairs studied so far. Between the giant fibrillar transcripts small transcripts can be observed, which usually display a distinct size gradient (Figure 2). We therefore assume that in this loop, secondary initiation sites exist within the transcription unit. This is in accord with data from recombinant DNA studies (Huijser and Hennig, unpublished data) and immunological evidence (Hulsebos *et al.*, 1984).

Comparison of the transcripts in three lampbrush chromosome loops

With neither of the two lampbrush loop pairs studied here did we succeed in spreading an entire loop in its full length. A desired spreading condition would be such that the transcripts covering the entire loop can be distinguished in order to establish their number and distances. The comparison of transcripts of the three loops described so far ('nooses': Grond *et al.*, 1983; 'threads' and 'pseudonucleolus': this paper) allows us to assess the reasons for the difficulties of such an approach. From a comparison of the morphology it is evident that the transcripts in the 'threads' and the 'pseudonucleolus' must be much longer than those in the 'nooses'. For the 'nooses' a minimum length of the transcripts of 260 kb has been derived from the size of the entire loop (Grond *et al.*, 1983). This implies that the sizes of the transcription units in the other two loops must be considerably larger than in the 'nooses'. We tried to measure the length of transcripts as shown in Figure 2 by adding up all linear sections and arrived at an estimate of at least 1500 kb for the 'pseudonucleolus' (for details of the estimates see Grond *et al.*, 1983). The transcription unit in the 'threads' may range between 500 and 1000 kb. It appears almost impossible to obtain fully spread loops of such a size in the electron microscope with current methodology. Also, the length of the individual transcripts, which could provide estimates on the length of the transcription unit, cannot be measured reliably because of their complicated secondary structure.

Discussion

Loop specificity of the transcripts

In this and an earlier study (Grond *et al.*, 1983) the transcript morphology of three of the five lampbrush chromosome loop pairs of the Y chromosome in *D. hydei* has been studied. The general conclusions for all three loop pairs are identical. All loops are giant transcription units with a high degree of secondary structure in their transcripts. The transcripts of all three loops display a characteristically different morphology, which permits us to identify their individual chromosomal origin. Part of the characteristic secondary structure may be due to intramolecular base pairing in the transcripts. The occurrence and distribution of inverted repeats in the DNA of the loop pair 'nooses' (Vogt *et al.*, unpublished data) would allow its transcripts to base pair intramolecularly. However, the highly characteristic transcript morphology for each of the loops is evidently determined by protein interaction with the RNA molecules. In the 'nooses' and the 'pseudonucleolus' many granular regions are seen in the transcripts, while the transcripts of the 'threads' lack them. In the latter the long linear RNA section is covered with protein, as the diameter of this portion of the transcript indicates (Figure 1), while the thinner bush-like part is less strongly coated with proteins.

It appears that initiation of transcription in the 'threads' and the 'pseudonucleolus' occurs less frequently and in a relatively irregular pattern compared with the 'nooses', a phenomenon which has more often been observed for non-ribosomal transcripts (Hamkalo *et al.*, 1973). However, it is clear from autoradiography that spermatocytes of stages Stc III and Stc IV have a strongly reduced transcriptional activity (Hennig, 1967) and probably one selects for loop sections with fewer transcripts since they can be more reliably recognized. Usually large areas of spread chromatin are not accessible to further analysis because of their complex structure. In comparing our spreads with the partially unfolded loops shown by Glätzer and Meyer (1981) we conclude that the 'threads' in earlier spermatocyte development have a much higher transcript density. It is remarkable that the time required to complete transcripts initiated at the beginning of stage Stc III is just the length of this stage ($\sim$20 h) (Hennig, 1967). Stage Stc III may therefore essentially serve for the completion of initiated transcripts. The transcript density during such a declining transcriptional activity should be lower than in earlier spermatocyte stages.

A special property of the 'pseudonucleolus' is the occurrence of transcripts strikingly different in size within short distances on the DNA axis (Figure 2). Two observations argue against this being the result of degradation. First, an alternative pattern of long and short transcripts has never been observed for any of the other two loops studied. An unspecific degradation of the RNA should affect all transcripts to some degree. Second, the small transcripts typically display a size gradient which probably indicates progressive steps of transcription. One would also expect a greater variability in the size of adjacent transcripts than is actually observed if degradation of the transcripts during spreading occurs regularly. We therefore believe that the presence of small transcripts between the giant transcripts in the 'pseudonucleolus' reflects secondary initiation of RNA synthesis within the transcription unit. Independent evidence for the presence of multiple initiation sites in the 'pseudonucleolus' has recently been obtained from immunological studies (Hulsebos *et al.*, 1984).

Relationship between transcripts and loop morphology

The loop pairs 'threads' and 'pseudonucleolus' have a complex morphology (see Grond *et al.*, 1984). Large portions of the loops contain hardly any nucleic acids (Grond *et al.*, 1984) but are rich in basic proteins (Kremer, 1983; cf. Grond, 1984). Such regions cannot, therefore, be composed of the giant transcripts. Other loop sections contain 35–40 nm ('threads') or 25–30 nm ('pseudonucleolus') particles which are considered as ribonucleoprotein particles (for details, see Grond *et al.*, 1984). Such loop sections should accommodate the transcripts. The relevant region in the 'threads' is the diffuse matrix or in the 'pseudonucleolus' the fibrillar matrix. For the 'threads' this localization of the transcripts could be confirmed by transcript *in situ* hybridization (Huijser and Hennig, in preparation).

The complex structure of the Y chromosomal transcripts together with ultrastructural and immunological data leads us to assume that the Y chromosomal transcripts have functions other than coding for proteins (see Hennig, 1984; Grond, 1984). The accumulation of distinct proteins in the nucleus could be such a function (see also Hulsebos *et al.*, 1984).

Materials and methods

Drosophila strains

Males with only the lampbrush chromosome loop pair 'threads' were obtained by mating $\bar{X}\bar{X}$/O females (Beck, 1976) to T(X;Y)6/Y males (Hackstein *et*

al., 1982). The X-Y translocation chromosome of these males carries only the distal part of the long arm of the Y chromosome with complementation group A, which is correlated with the expression of the 'threads'. Males with only the loop pair 'pseudonucleolus' were obtained by crossing $\widehat{XX}$/O females to T(X;Y)74/Y males. The Y translocation includes the distal part of the long arm of the Y with complementation groups A–G. Complementation group A is inactive in this strain. Since complementation group C is correlated with the expression of the 'pseudonucleolus' and the other loci do not form prominent loops, the 'pseudonucleolus' is the only large lampbrush chromosome loop in the T(X;Y)74/O males. The character of the 'cones', which are attached to the 'pseudonucleolus', remains uncertain (for discussion, see Hennig, 1967, 1984; Grond *et al.*, 1984). However, since the 'cones', which are present in both translocations used in our experiments, are minute compared with the other lampbrush chromosome loops, and since they are expected to display the same type of transcripts in both strains in case they represent a separate transcription unit, they can be neglected in the present experiments. The 'cones' are expected to have small transcripts as they are frequently found (Grond *et al.*, 1983).

Cytology

Cytological preparations were made as described before (Grond *et al.*, 1983).

Chromatin spreading according to Miller

Spreading experiments were carried out as described earlier (Grond *et al.*, 1983).

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